Supplementary Appendix

Supplement to: Letizia AG, Ramos I, Obla A, et al. SARS-CoV-2 transmission among Marine recruits during quarantine. N Engl J Med. DOI: 10.1056/NEJMoa2029717

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: SARS-CoV-2 transmission among Marine recruits during quarantine.

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Supplemental Methods

ELISA Assays

The presence of IgG SARS-CoV2 Spike (S) receptor binding domain (RBD) specific antibodies in serum was evaluated using an enzyme-linked immunosorbent assay (ELISA) as previously described [1] with some modifications: 384-well Immulon 4 HBX (Thermofisher) plates were coated with recombinant His-tagged S-RBD (SinoBiological, 2 µg/ml); peroxidase conjugated goat F(ab')2 Anti-Human IgG (abcam) was used for IgG antibody detection. After assay development (SIGMAFAST™ OPD followed by 3M HCl), optical density (OD) at 492 nm was determined. At least 2 positive controls, 8 negative controls (sera collected before July 2019) and 4 blanks (no serum) were included in every plate. All serum samples were first screened at a 1:50 dilution. Those samples with OD 492 nm value higher than the average of the negative controls plus 3 times their standard deviation (SD) underwent titration assay (6 serial 1:3 serum dilutions starting at 1:50). Serum samples were considered positive when at least 2 consecutive dilutions showed higher OD 492 nm than the greater of the average of the negative controls plus 3 times their SD at the correspondent dilution or 0.15 OD 492 nM.

SARS-CoV-2 whole-genome sequencing

The Illumina sequencing-based protocol involved cDNA synthesis, targeted PCR amplification, and Nextera XT library preparation, using a custom tiling primer design and the Artic Consortium protocol (https://artic.network/ncov-2019), as previously described [2]. For IonTorrent sequencing, up to 100ng of RNA was used as input for a cDNA reaction with the NEB Protoscript II First Strand Synthesis Kit (E6560) using random hexamer primers, according to the manufacturer's instructions. The Thermo Fisher Scientific (TFS) Ion AmpliSeq SARS-CoV-2 Research Panel and the Ion AmpliSeq Library Kit Plus (TFS A35907) were then used to generate amplicon libraries. After barcoding using the IonCode Barcode Adapters 1-384 Kit (TFS A29751), quantification with the Ion Library Taqman Quantification Kit (TFS 4468802), and templating on the Ion Chef Instrument (TFS 4484177), sequencing was performed on the Ion Genestudio S5 System (TFS A38194) using 500 flows per chip. Samples were templated and sequenced using the Ion 530 Chip Kit (A27764) or the Ion 540 Chip Kit (TFS A27765).

Phylogenetic analysis

Global background SARS-CoV-2 genomes and associated metadata were downloaded from the GISAID EpiCoV database [3] on August 11, 2020 (79,840 sequences) and sampled using the default NextStrain [4] sub-sampling scheme to maximize representation of USA genomes. The resulting 11,434 background genomes were analyzed together with the 36 genomes obtained in this study with >95% completeness. Phylogenetic analyses were performed using the v1.0-292-ga9de690 Nextstrain build for SARS-CoV-2 genomes with default parameters. Identification of transmission/outbreak events was based on co-clustering of SARS-CoV-2 genomes from study subjects in monophyletic clusters within the NextStrain phylogenetic tree visualized with TreeTime [5], as well as comparative analysis of mutation profiles relative to the SARS-CoV-2

Study Contributions

	Andrew G. Letizia	Irene Ramos	Ajay Obla	Carl Goforth	Dawn L. Weir	Yongchao Ge	Marcas M. Bamman	Jayeeta Dutta	Ethan Ellis	Luis Estrella	Mary-Catherine George	Ana S. Gonzalez-Reiche	William D. Graham	Adriana van de Guchte	Ramiro Gutierrez	Franca Jones	Aspasia Kalomoiri	Rhonda Lizewski	Stephen Lizewski	Jan Marayag	Nada Marjanovic	Eugene V. Millar	Venugopalan D. Nair	German Nudelman	Edgar Nunez	Brian L. Pike	Chad Porter	James Regeimbal	Stas Rirak	Ernesto Santa Ana	Rachel S. Gelernter Sealfon	Robert Sebra	Mark P. Simons	Alessandra Soares-Schanosk	Victor Sugiharto	Michael Termini	Sindhu Vangeti	Carlos Williams	Olga G. Troyanskaya	Harm van Bakel	Stuart C. Sealfon
Study Design	X	X		X	X	X	X				X				X	X																						X		X	X
Data Gathering	X	X		X	X			X	X	X			X	X			X	X	X	X	X	X	X		X	X		X		X		X	X	X	X	X	X				
Data Analysis	X	X	X	X	X	X						X												X					X		X		X							X	X
Writing	X	X	X	X	X	X	X					X											X				X				X		X						X	X	X
Decision to Publish	X																																							X	X

The authors accept full responsibility for the conduct of the study, had access to the data, and controlled the decision to publish.

Participants First qPCR Positive on Day 0

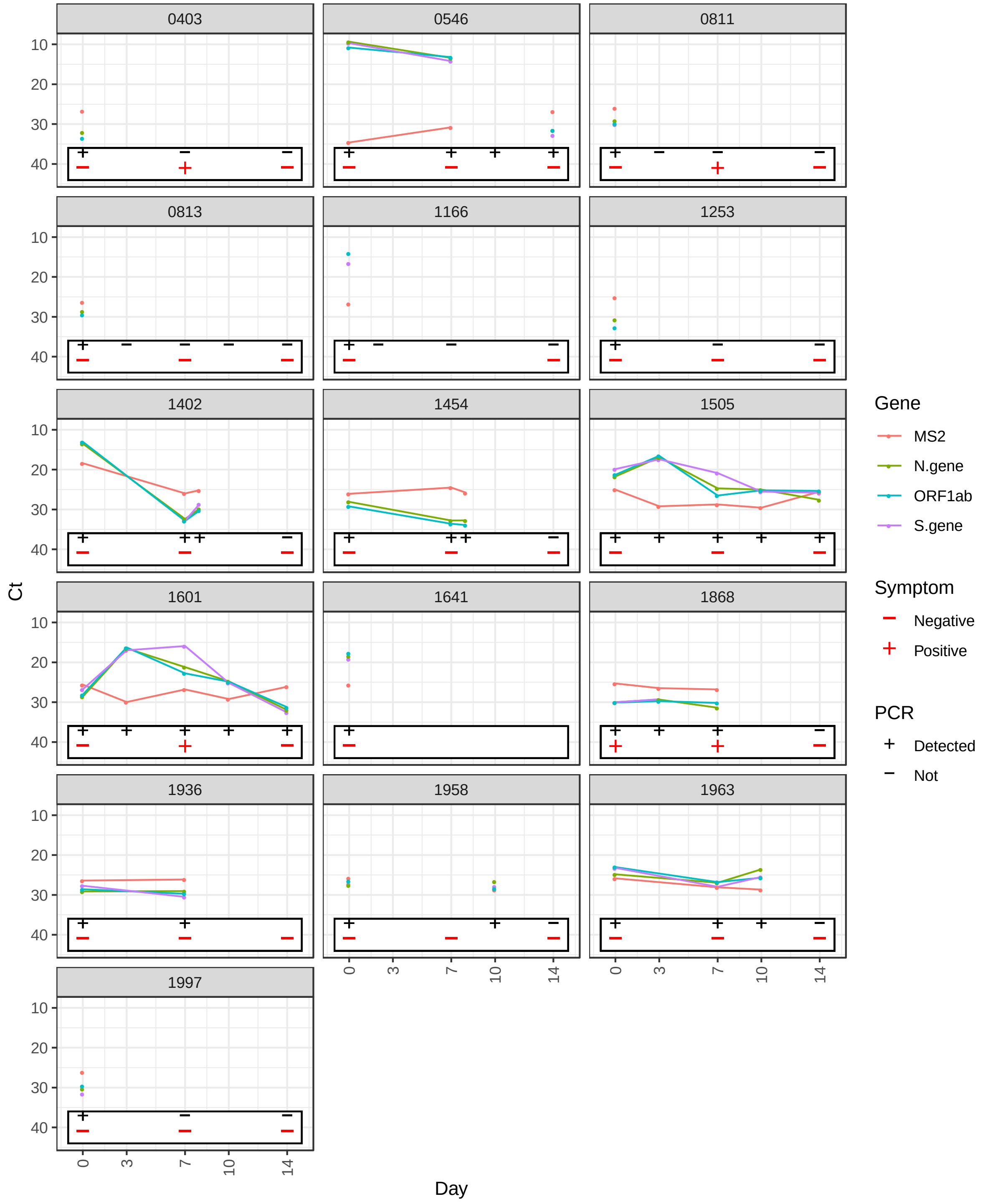

Figure S1. SARS-CoV-2 qPCR Probe Ct Values and Symptoms over Time in 51 Infected Participants

a. Presence of any symptoms (red + or -) and result of the SARS-CoV-2 qPCR diagnostic test (black + or -) in 16 participants first positive at day 0 are indicated. Symptom assessment at time day 0 reflects presence of symptoms at enrollment or during the prior seven days. The assessments at day 7 and at day 14 represent the period since the previous assessment. The Ct levels for the three SARS-CoV-2 targets (N-gene, ORF1ab, S gene) as well as the positive assay control (MS2) are shown. In some instances, the overall test result, but not the probe Ct values, was available.

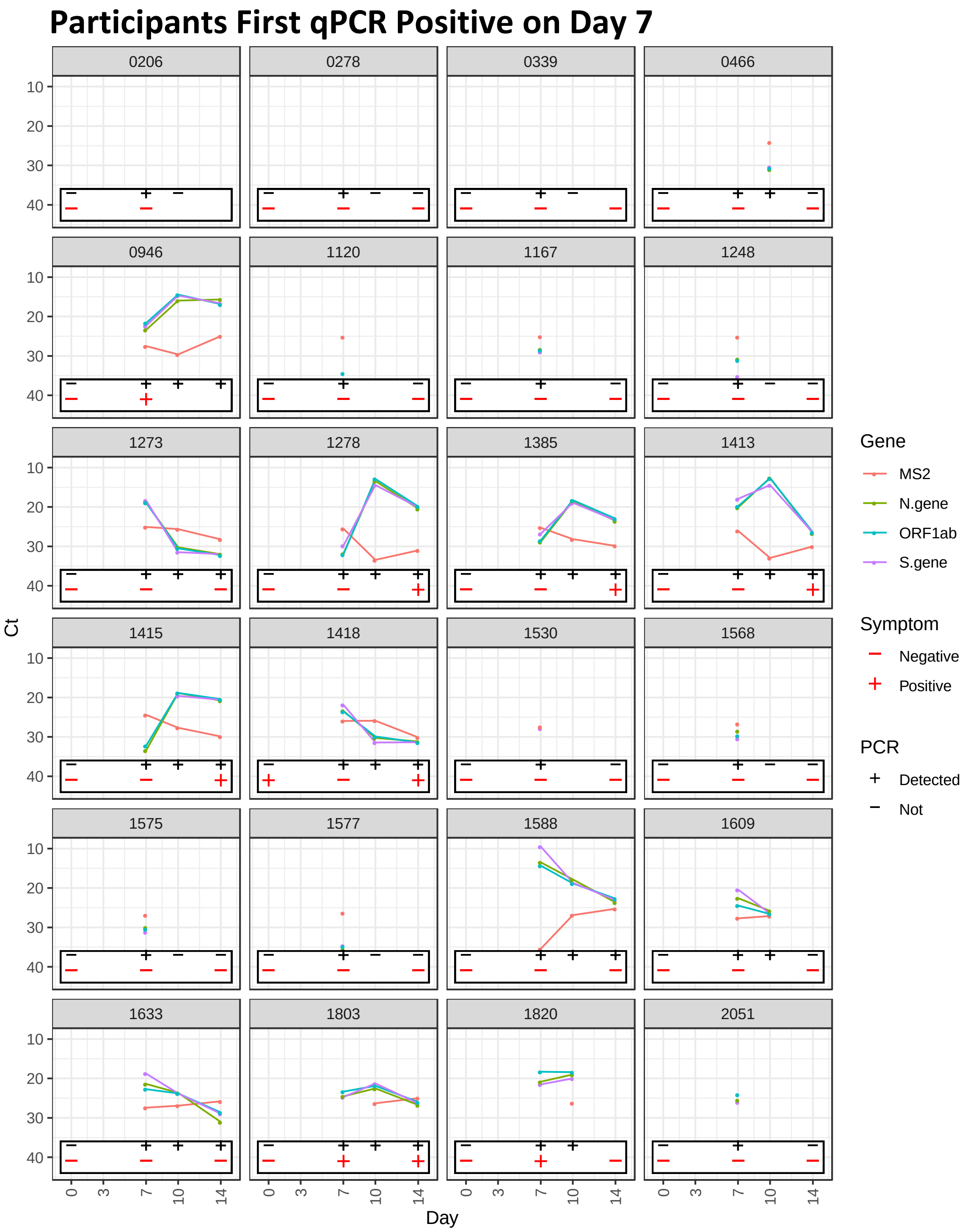

Figure S1. SARS-CoV-2 qPCR Probe Ct Values and Symptoms over Time in 51 Infected Participants

b. Presence of any symptoms (red + or -) and result of the SARS-CoV-2 qPCR diagnostic test (black + or -) in 24 participants first positive at day 7. Symptom assessment at time day 0 reflects presence of symptoms at enrollment or during the prior seven days. The assessments at day 7 and at day 14 represent the period since the previous assessment. The Ct levels for the three SARS-CoV-2 targets (N-gene, ORF1ab, S gene) as well as the positive assay control (MS2) are shown. In some instances, the overall test result, but not the probe Ct values, was available.

Participants First qPCR Positive on Day 14

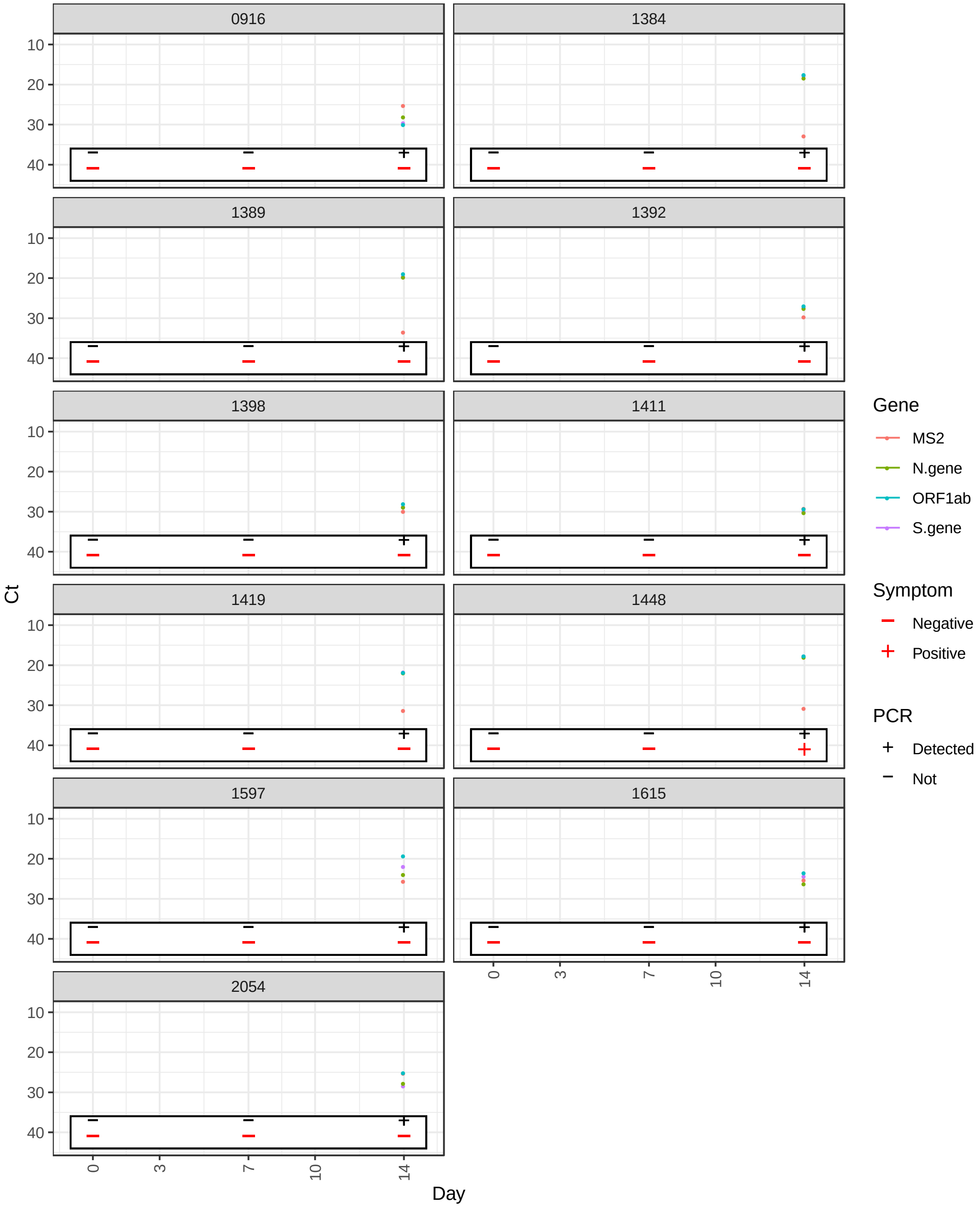

Figure S1. SARS-CoV-2 qPCR Probe Ct Values and Symptoms over Time in 51 Infected Participants

c. Presence of any symptoms (red + or -) and result of the SARS-CoV-2 qPCR diagnostic test (black + or -) in 11 participants first positive at day 14. Symptom assessment at day 0 reflects presence of symptoms at enrollment or during the prior seven days. The assessments at day 7 and at day 14 represent the period since the previous assessment. The Ct levels for the three SARS-CoV-2 targets (N-gene, ORF1ab, S gene) as well as the positive assay control (MS2) are shown.

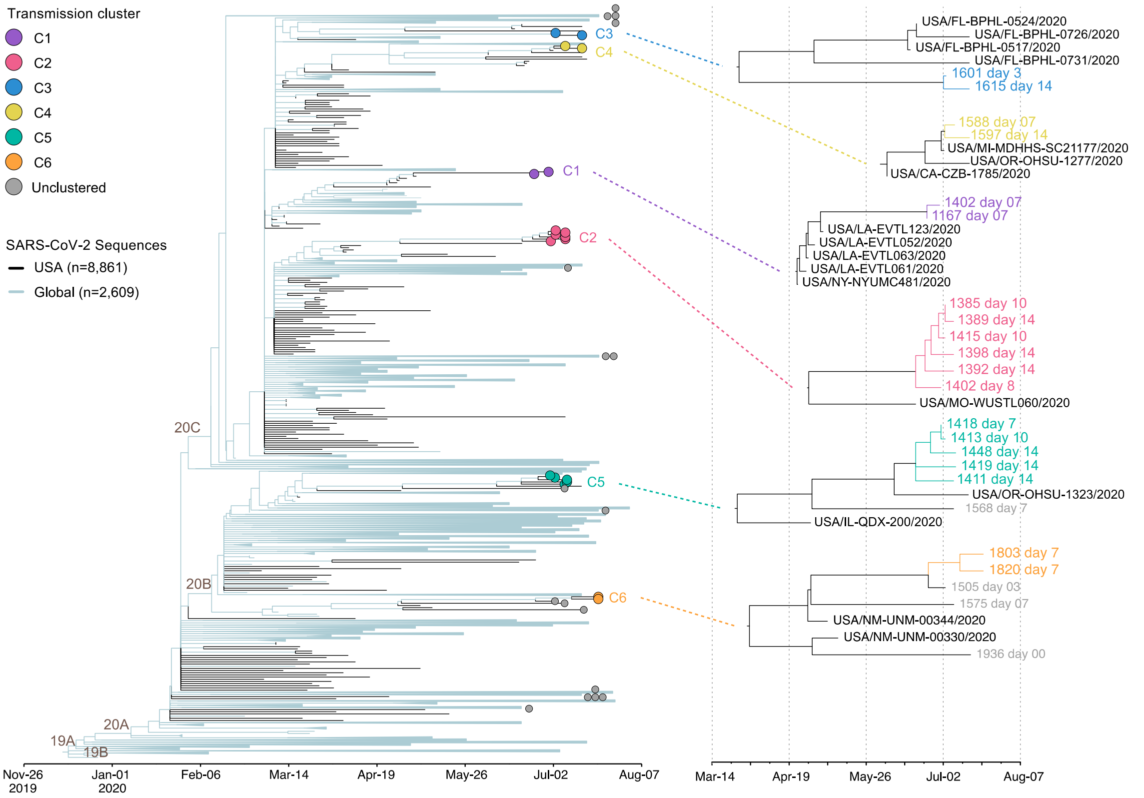

Figure S2. SARS-CoV-2 Introductions and Transmission Clusters.
Time-scaled phylogenetic relationships of 36 SARS-CoV-2 isolates from the CHARM study and a USA (black branches) and global background (light blue branches) of 11434 sequences from GISAID between December 2019 and August 2020. Clades with only background sequences or CHARM isolates outside of transmission clusters are collapsed for visualization. The positions of the 32 recruits are indicated by the circles at the tips of the branches and colored by transmission cluster. CHARM isolates outside of transmission clusters are shown in grey, where stacked circles represent longitudinal samples from the same individuals. The detailed transmission clusters are shown on the right.

Table S1. Public Health Practices Used Throughout the Quarantine Period to Mitigate Spread of SARS-CoV-2.

Home Quarantine	Recruits instructed to self-quarantine at home for two weeks prior to travel to campus for supervised quarantine
	Instructed to wear masks at all times and social distance during travel to campus
Supervised Quarantine Site	Isolated college campus used solely for supervised quarantine
	Recruit classes that overlap (typically by one week) housed in separate dorms
Personal Protective Measures	Everyone wore double-layer cloth masks except during meals and sleeping
	Social distancing of at least 6 feet at all times except for roommates in quarters
	Supervised hand hygiene with soap and water before and after meals
	Hand sanitizer dispenser stations at doors of buildings and in all dorm corridors
	Pre-plated meals
	Recruits wiped down shower stall with bleach wipes after each use
	Instruction and training performed outdoors whenever possible
Cohorting and Movement Control	Recruits restricted to room when not with instructor; hallways monitored at night
	Rooms limited to two recruits
	Same training partner, usually roommate, throughout quarantine unless in isolation
	No gatherings in common spaces; congregating not permitted
	Instructors do not leave campus or interact with local community
	Instructors ensured no interactions between recruits and campus staff
	Meal times between platoons and classes staggered
	Orderly, supervised unidirectional flow of platoons indoors and between activities
Cleaning and Disinfection	Bleach sanitation of all surfaces in rooms, showers, toilets, sinks and dining halls
	At end of quarantine, rooms thoroughly cleaned by recruits, then professionally sprayed with atomized bleach solution, and left vacant at least 72h.
Testing and Contact Investigation	Clinically indicated qPCR testing for SARS-CoV-2 of recruits and instructors with subsequent isolation if positive
	qPCR SARS-CoV-2 testing of instructors with an infected recruit in their platoon
	Contact tracing of high-risk exposures for quarantine and isolation as needed
	Daily temperature checks and symptom screening for recruits and instructors
Risk Communication and Education	Upon arrival at quarantine, instructors and recruits formally educated on COVID-19 symptoms, signs, prevention and mitigation strategies
	Informational signage posted throughout campus to emphasize public health measures, direct traffic flow, and label quarantine and isolation areas

Table S2. **Viral Load (Ct Values) in Symptomatic and Asymptomatic Participants at Diagnosis**

	Symptomatic participants						Asymptomatic participants						Ct difference	
gene	**n**	**mean**	**sd**	**median**	**min**	**max**	**n**	**mean**	**sd**	**median**	**min**	**max**	**mean1-mean2**	**median1-median2**
S.gene	5*	23.27	4.55	22.38	17.68	30.02	35*	24.53	6.29	26.77	9.49	35.20	-1.26	-4.39
N.gene	5*	23.36	4.48	23.40	18.02	30.00	39*	25.56	5.85	27.70	9.33	35.62	-2.20	-4.3
MS2†	3*	27.87	2.77	27.51	25.30	30.80	41*	26.91	3.06	26.02	18.40	35.45		
ORF1ab	5*	22.20	5.00	21.66	17.61	30.09	41*	25.30	6.23	26.88	10.81	34.80	-3.10	-5.22

*All probes were not detected in all positive samples.

†Positive Control

Table S3. Transmission Clusters Identified by SARS-CoV-2 Mutation Profile

		Distinguishing Mutation Profile [Relative to Wuhan Reference Genome]															
Participant	Cluster	[G] 10262	[A] 11411	[A] 11740	[C] 14408	[G] 17298	[A] 18424	[G] 18951	[G] 19656	[C] 21304	[G] 21578	[C] 24865	[A] 25336	[G] 25907	[C] 26256	[C] 28472	[C] 28869
1167	C1	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-
1402	C1	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-
1402	C2	-	-	-	T	T	-	-	-	-	N	T	-	-	-	-	-
1415	C2	-	-	-	T	T	-	-	-	-	-	T	-	-	-	-	-
1385	C2	-	-	-	T	T	-	-	-	-	-	T	-	-	-	-	-
1389	C2	-	-	-	T	T	-	-	-	-	-	T	-	-	-	-	-
1392	C2	-	-	-	T	T	-	-	-	-	-	T	-	-	-	-	-
1398	C2	-	-	-	T	T	-	-	-	-	-	T	-	-	-	-	-
1601	C3	T	G	-	T	-	-	T	T	-	-	-	-	-	-	-	-
1615	C3	T	G	-	T	-	-	T	T	-	-	-	-	-	-	-	-
1588	C4	-	-	-	T	-	G	-	-	T	-	-	-	T	-	T	T
1597	C4	-	-	-	T	-	G	-	-	T	-	-	-	T	-	T	T
1418	C5	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-
1413	C5	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-
1411	C5	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-
1419	C5	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-
1448	C5	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-
1820	C6	-	-	G	T	-	-	-	-	-	T	-	-	-	T	-	-
1803	C6	-	-	G	T	-	-	-	-	-	T	-	-	-	T	-	-

Table S4. Complete Mutation Profile of All Sequenced Isolates Including Clade-Defining Variants

Participant Data					Mutation Profile [Relative to Wuhan Ref Genome]																																								
Participant	Day	Clade	Cluster	SARS-CoV-2 on arrival	[C] 241	[C] 601	[C] 811	[C] 815	[T] 833	[C] 936	[C] 1059	[C] 1385	[T] 1728	[G] 1738	[G] 1748	[C] 3037	[A] 3054	[G] 3242	[G] 3259	[C] 3411	[C] 3828	[G] 4148	[G] 4288	[C] 5547	[T] 7153	[A] 7993	[G] 8083	[C] 8139	[C] 8389	[A] 8845	[C] 8950	[G] 10262	[C] 10319	[C] 10376	[T] 10795	[G] 11083	[A] 11411	[A] 11740	[C] 11916	[G] 12832	[C] 14408	[C] 14805	[C] 14937	[G] 15438	[C] 16260
546	0	20C	U	+	T	-	-	-	-	-	T	-	-	-	-	T	G	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	T	-	-	-	-
946	7	20C	U	−	T	-	T	-	-	-	T	-	-	-	A	T	-	-	-	-	-	-	T	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	T
946	10	20C	U	−	T	-	T	-	-	-	T	-	-	-	A	T	-	-	-	-	-	-	T	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	T
946	14	20C	U	−	T	-	T	-	-	-	T	-	-	-	A	T	-	-	-	-	-	-	T	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	T	-	-	-	T
1167	7	20C	C1	−	N	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1402	7	20C	C1	+	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1278	7	20C	U	−	T	-	N	N	N	-	T	T	-	-	-	T	-	A	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	T	-	-	-	-
1273	7	20C	U	−	T	-	-	-	-	-	T	T	-	T	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	T	-	-	-	-
1402	8	20C	C2	+	T	-	-	-	C	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1415	10	20C	C2	−	T	-	-	-	C	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1385	10	20C	C2	−	T	-	-	-	C	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1389	14	20C	C2	−	T	-	-	-	C	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1392	14	20C	C2	−	T	-	-	-	C	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1398	14	20C	C2	−	T	-	-	-	C	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1601	3	20C	C3	+	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	T	-	-	-	G	-	-	-	T	-	-	-	-
1615	14	20C	C3	−	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	T	-	-	-	G	-	-	-	T	-	-	-	-
1588	7	20C	C4	−	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	T	-	-	-	-	-	-	-	T	T	-	-	-
1597	14	20C	C4	−	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	T	-	-	-	-	-	-	-	T	T	-	-	-
1633	7	20C	U	−	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1418	7	20B	C5	−	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	G	-	T	-	-	-	-	-	T	-	-	-	-	-	-	T	-	-	-	-
1413	10	20B	C5	−	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	G	-	T	-	-	-	-	-	T	-	-	-	-	-	-	T	-	-	-	-
1411	14	20B	C5	−	N	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	G	-	T	-	-	-	-	-	-	-	-	-	-	-	-	T	-	T	-	-
1419	14	20B	C5	−	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	G	-	T	-	-	-	-	-	T	-	-	-	-	-	-	T	-	T	-	-
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1568	7	20B	U	−	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	G	-	T	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1505	3	20B	U	+	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-
1820	7	20B	C6	−	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	T	-	-	-	-	G	-	-	T	-	-	-	-
1803	7	20B	C6	−	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	T	-	-	-	-	G	-	-	T	-	-	-	-
1575	7	20B	U	−	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	T	-	-	-	-
1936	0	20B	U	+	T	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1609	7	20B	U	−	T	-	-	T	-	-	-	-	-	-	-	T	-	-	-	T	-	T	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	T	-	-	-	-
1577	7	20A	U	−	T	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1868	0	20A	U	+	T	T	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	T	T	-	-	-	-
1958	0	20A	U	+	N	-	-	-	-	-	T	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	T	-	-	T	-
1963	0	20A	U	+	N	-	-	-	-	T	-	-	-	-	-	T	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-
1963	7	20A	U	+	N	-	-	-	-	T	-	-	C	-	-	T	-	-	-	-	N	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-

Participant	[C] 16349	[A] 16373	[C] 16616	[G] 16710	[G] 17298	[C] 17822	[A] 18424	[C] 18452	[G] 18951	[T] 19653	[G] 19656	[G] 20176	[G] 20610	[C] 21110	[C] 21304	[G] 21578	[G] 21641	[G] 22021	[T] 22031	[T] 22287	[A] 23403	[C] 23707	[T] 24274	[C] 24865	[A] 25121	[A] 25201	[A] 25336	[G] 25437	[G] 25563	[G] 25907	[C] 26256	[G] 27238	[G] 27915	[C] 27964	[G] 28044	[C] 28087	[C] 28472	[C] 28821	[C] 28846	[C] 28869	[A] 28877	[G] 28881	[G] 28882	[G] 28883
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